STATISTICAL STUDY OF THE RELATIONSHIPS OF BASEMENT MEMBRANE AND EPIDERMIS VARIATIONS IN COWHIDES*

Edward A. Botan†

AND

Albert E. Chouinard

with the technical assistance of

Mrs. Nadja Bisaillon

Lowell Technological Institute
Lowell, Mass.

ABSTRACT

The thickness of the basement membrane and epidermis in a cowhide was determined in the belly, butt, backbone, shank, and shoulder areas using sections stained with periodic acid Schiff reagent (P.A.S.). It was found that the basement membrane and epidermis vary in thickness in the different areas of the hide. The left side of the hide was shown to have a pattern of thickness variations almost identical to that of the right side of the hide. The maximum thickness for the basement membrane (unfixed sections) was found in the belly area, 11.71 μ, and the minimum was found in the shoulder, 10.01 μ. The maximum thickness for the epidermis was found in the belly area, 32.07 μ, and the minimum thickness was found in the shoulder, 24.9 μ. In fixed sections the thickness of the epidermis varies from 25.86 μ to 27.37 μ, and the thickness of the basement membrane varies from 10.01 μ to 10.95 μ. As the thickness of the basement membrane was found to increase or decrease, a similar change was observed in the epidermis.

It is suggested that in unhairing and in the removal of the epidermis from hides and skin, the thicknesses of the basement membrane and the epidermis, their ratio, and the chemical composition of the basement membrane are important factors.

INTRODUCTION

The basement membrane is an area between the epidermis and the grain (corium minor). It consists of amorphous, fibrous, and cellular elements. The amorphous material is mainly mucoprotein, with possibly some glycoprotein; the fibers are elastin and reticular; and the cells are fibroblasts (few in number). The basement membrane is an undulating structure which dips into the corium minor, following the interpapillary pegs and hair follicles. The elastin and reticular fibers can be traced from the basement membrane into the grain (corium minor). Photomicrographs of these structures and the techniques used in their staining can be seen in Everett and Cordon (1). It can therefore be assumed that the basement membrane has an important role in anchoring the epidermis to the dermis and the hair follicles to the dermis. Thus, it was of interest to examine the variations in thickness of the epidermis and the basement membrane and attempt to relate this information to the process of unhairing (removing the hair and epidermis from a hide).

MATERIALS AND METHODS

Hide samples.—The hide samples used in this study came from heavy cowhides obtained from a local abattoir. After the animal was slaughtered, the hide was cooled, sided, trimmed, washed, and placed in a deep freeze. Strips of the hide were removed from the freezer and used as needed. Before the hide samples were placed in fixative, they were hand-fleshed and cut into blocks about ½″ x ½″.

Fixatives.—Formal saline and Lillie's (2) alcoholic lead nitrate were used.

Sectioning.—Blocks of fixed or unfixed cowhide were washed in distilled water. The block of hide was placed on the stage of a sliding freezing-microtome, a combination of a model 680 Spencer sliding microtome with an attached Histofreeze freezing-head. Vertical sections of the cowhide were then cut 6–8 μ thick. Each section contained epidermis, basement membrane, and dermis (coriums, minor and major).

Staining.—The P.A.S. (periodic acid Schiff) stain was carried out according to the methods of Hotchkiss (3) and Pearse (4). Pearse's modification for P.A.S. stain was found to give better results than the Hotchkiss technique.
  A more complete histochemical examination of the basement membrane is being prepared for publication elsewhere by the authors.

Measuring the basement membrane and epidermis.—A Filar micrometer calibrated by means of a stage micrometer was used for thickness measurements. All measurements were reported in micra.

Sampling areas.—The hide was cut into sides, left and right. Five locations were sampled in each side: butt, belly, backbone, shank, and shoulder (Fig. 1). Each of the sampling areas was 5″ x 10″.
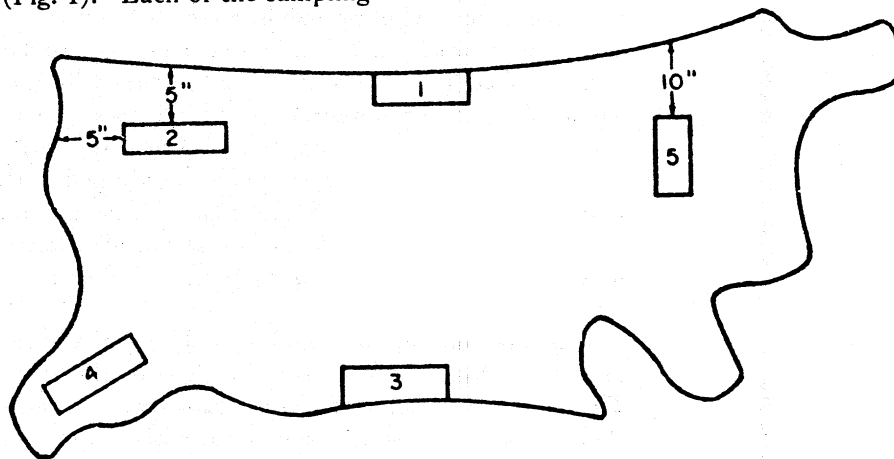

FIGURE 1.—Sampling locations for statistical analysis of the cowhide, representative of both sides.

1 = backbone
2 = butt
3 = belly
4 = shank
5 = shoulder

Determination of the level of significance and the number of observations.—Sample area No. 1, backbone, was cut into 100 blocks, and 25 were selected at random. One section from each of the 25 blocks was selected and stained with the P.A.S. stain. The thickness of the epidermis and basement membrane was measured at two places in each section. The average values for each section were used in the calculations.

The standard deviation (σ) was calculated by using the following:

$$S.\,D. = \left[\frac{\Sigma m \ (Am - \overline{A})^2}{m \ (m - 1)} \right]^{\frac{1}{2}}$$

where Am = individual measurements
$\overline{A}$ = arithmetic mean
m = number of measurements
Σm = summation of m differences

The standard error (S.E.) was obtained by using the formula:

$$S.E. = \frac{\text{standard deviation}}{\sqrt{n}}$$

where n equals the number of samples, in this case 25. The standard deviation

was corrected to allow for the sample size. The C_2 value was obtained from the A. S. T. M. Manual on Quality Control of Materials (5).

The variance was next calculated from $V = \dfrac{\sigma}{\overline{X}}$, ($\overline{X}$ = mean). By using this result the level of probability and the number of tests were chosen, using a sampling error level of 5% (6). The decision to use a sampling error of 5% and a probability level of 95% was governed by a practical consideration of the level of accuracy desired versus the reasonable number of sections that would be necessary for that degree of probability in view of the time factor.

The first backbone section which was studied in this manner was cut from a black-haired cowhide (Black Angus). The amount of pigmentation in the critical area gave a statistical evaluation of n (the number of blocks to be used in any location of the skin) of 25, at a 95% probability level and a sampling error of 5%.

This large number of samples would have required a considerable amount of time to study each location in view of the lengthy staining procedures. The extensive amount of pigmentation was also a disturbing factor. It was therefore decided to abandon the use of this skin for this particular study, and a new study was begun using a cowhide which was light brown with white patches.

The results of this evaluation of P and n are shown in Table I. Using the same 95% probability graph and the new percent variability, the 5% sampling error curve gave a value for n, the total number of samples to be taken, of 12.

The other four locations in this side were then evaluated on the basis of of 12 randomly selected blocks from each location, and the data are indicated in Table II.

The aforementioned procedure was used in the study of the five areas of the right side of the hide. When the statistical analysis of the right side of the hide was completed, it was decided to reduce the number of blocks used in the study of the left side to eight blocks from each area. These eight blocks were further subdivided to four fixed and four unfixed in order to analyze the effects of fixation on the thickness of the various components of the sections. The standard deviation was again corrected for sample size, and the new C_t value was taken from the A. S. T. M. Manual on Quality Control (5).

RESULTS

Tables I and II present the variations in thickness of epidermis and basement membrane in two places in each section chosen as representative of each block for each location.

Table III indicates the variations obtained between fixed and unfixed sections in each area of the left side.

Table IV summarizes the over-all statistical information for each location.

TABLE I

THICKNESS OF EPIDERMIS AND BASEMENT MEMBRANE IN BACKBONE LOCATION

(*Fixed Sections from Right Side*)

Block No.	Epidermis, Micra a*	b*	Basement Membrane, Micra a*	b*	Block No.	Epidermis, Micra a*	b*	Basement Membrane, Micra a*	b*
1	27.18	28.69	10.57	10.57	14	27.18	28.69	10.57	10.57
2	33.22	30.20	9.06	10.57	15	28.69	30.20	9.06	9.06
3	30.20	28.69	12.08	12.08	16	31.71	30.20	10.57	12.08
4	28.69	30.20	9.06	10.57	17	25.67	27.18	12.08	10.57
5	27.18	25.67	12.08	10.57	18	28.69	25.67	12.08	10.57
6	27.18	28.69	10.57	10.57	19	28.69	30.20	10.57	10.57
7	28.69	28.69	12.08	10.57	20	28.69	27.18	9.06	10.57
8	27.18	27.18	10.57	12.08	21	27.18	28.69	12.08	10.57
9	30.20	28.69	12.08	12.08	22	25.67	28.69	10.57	9.06
10	27.18	27.18	10.57	10.57	23	27.18	24.16	7.55	10.57
11	30.20	27.18	10.57	10.57	24	27.18	28.69	9.06	9.06
12	28.69	30.20	13.59	12.08	25	27.18	27.18	12.08	10.57
13	28.69	28.69	12.08	10.57					

N. B. The data from this location were used to determine the level of uncertainty (P) and the number of samples (n).
* a and b represent two readings from the same section.

TABLE II

THICKNESS OF EPIDERMIS AND BASEMENT MEMBRANE

(*Fixed Sections from Right Side*)

Block No.	Epidermis, Micra Location* B	C	D	E	Basement Membrane, Micra Location* B	C	D	E
1a†	25.7	30.2	28.7	19.6	10.6	13.6	10.6	9.1
1b†	27.2	27.2	30.2	19.6	7.5	10.6	7.5	7.5
2a	30.2	33.2	19.6	18.1	7.5	9.1	7.5	9.1
2b	27.2	25.7	19.6	19.6	10.6	12.1	12.1	10.6
3a	18.1	28.7	21.1	18.1	9.1	7.5	9.1	10.6
3b	22.6	22.6	27.2	15.1	7.5	12.1	10.6	10.6
4a	27.2	40.8	2.11	15.1	9.1	12.1	9.1	9.1
4b	25.7	28.7	16.6	16.6	12.1	10.6	7.5	7.5
5a	21.1	22.6	24.2	18.1	7.5	7.5	13.6	9.1
5b	25.7	30.2	18.1	16.6	6.0	13.6	7.5	12.1
6a	30.2	37.7	34.7	18.1	6.0	15.1	10.6	7.5
6b	21.1	31.7	28.7	16.6	7.5	10.6	9.1	12.1

TABLE II (*Continued*)

Block No.	Epidermus, Micra Location*				Basement Membrane, Micra Location*			
	B	C	D	E	B	C	D	E
7a	21.1	33.2	24.2	18.1	9.1	12.1	12.1	9.1
7b	19.6	34.7	21.1	18.1	12.1	13.6	7.5	7.5
8a	23.6	37.7	27.2	16.6	7.5	13.6	10.6	10.6
8b	25.7	27.2	25.7	21.1	12.1	9.1	7.5	7.5
9a	21.1	28.7	22.6	24.2	6.0	10.6	9.1	9.1
9b	23.7	30.2	28.7	21.1	7.5	13.6	6.0	7.5
10a	21.1	48.3	21.1	21.1	12.1	9.1	13.6	12.1
10b	27.2	33.2	27.2	21.1	7.5	12.1	9.1	9.1
11a	23.6	28.7	28.7	21.1	9.1	13.6	9.1	7.5
11b	21.1	22.6	25.7	19.6	6.0	7.5	10.6	10.6
12a	30.2	27.2	21.1	18.1	10.6	10.6	9.1	9.1
12b	24.2	39.3	30.2	22.6	7.5	12.1	7.5	7.5

* B = Butt; C = Belly; D = Shank; E = Shoulder.
† *a* and *b* represent two readings from the same section.

TABLE III

THICKNESS OF EPIDERMIS AND BASEMENT MEMBRANE

(*Left Side*)

Block No.	Backbone		Butt		Belly		Shank		Shoulder	
	Fixed	Unfixed	Fixed	Unfixed	Fixed	Unfixed	Fixed	Unfixed	Fixed	Unfixed
Epidermis, Micra										
1a	30.2	24.2	25.7	24.2	27.2	43.6	30.2	30.2	24.2	21.1
1b	24.2	31.7	22.6	28.7	35.7	24.2	27.2	24.2	28.7	25.7
2a	22.6	22.6	24.2	30.2	31.7	25.7	34.7	24.2	22.6	25.7
2b	27.2	34.7	22.6	27.2	24.2	36.2	18.7	31.7	27.2	22.6
3a	27.2	27.2	25.7	28.7	22.6	31.7	25.2	28.7	25.7	31.7
3b	25.7	30.2	28.7	25.7	30.2	28.7	22.6	22.6	27.2	19.6
4a	30.2	25.7	30.2	22.6	28.7	39.3	40.7	27.2	24.2	22.6
4b	31.7	34.7	27.2	31.7	25.7	27.2	17.2	25.7	30.2	30.2
Basement Membrane, Micra										
1a	12.1	12.1	12.1	7.5	10.6	9.1	9.1	12.1	9.1	7.5
1b	10.6	9.1	9.1	12.1	9.1	13.6	13.6	10.6	12.1	12.1
2a	9.1	7.5	7.5	10.6	12.1	15.1	10.6	9.1	7.5	13.6
2b	10.6	13.6	10.6	9.1	7.5	7.5	12.1	12.1	10.6	9.1
3a	13.6	9.1	13.6	10.6	9.1	10.6	10.6	12.1	10.6	10.6
3b	12.1	15.1	9.1	12.1	10.6	12.1	9.1	9.1	9.1	9.1
4a	7.5	13.6	10.6	7.5	13.6	12.1	12.1	13.6	7.5	7.5
4b	12.1	10.6	12.1	13.6	9.1	13.6	7.5	7.5	13.6	10.6

TABLE IV

SUMMARY OF THICKNESS MEASUREMENTS OF EPIDERMIS AND BASEMENT MEMBRANE IN VARIOUS AREAS OF COWHIDE

Location in Hide	Epidermis			Basement Membrane		
	Mean Thickness, Micra	Standard Deviation	Standard Error	Mean Thickness, Micra	Standard Deviation	Standard Error
Fixed Sections—Right Side						
Backbone	28.3	1.42	0.284	10.8	0.99	0.198
Butt	24.3	2.68	0.774	8.7	1.45	0.417
Belly	3.13	4.62	1.33	11.3	0.978	0.279
Shank	24.7	3.82	1.12	9.4	1.05	0.303
Shoulder	18.9	2.032	0.586	9.2	0.978	0.279
Unfixed Sections—Left Side						
Backbone	28.9	1.03	0.515	11.3	0.95	0.475
Butt	27.4	1.04	0.52	10.4	0.78	0.39
Belly	32.1	1.93	0.965	11.7	0.83	0.415
Shank	26.8	1.06	0.53	10.8	0.41	0.25
Shoulder	24.9	1.50	0.75	10.0	1.03	0.515
Fixed Sections—Left Side						
Backbone	27.4	2.78	1.39	10.9	1.56	0.78
Butt	25.9	2.7	1.35	10.6	1.2	0.58
Belly	27.0	0.78	0.39	10.2	0.83	0.415
Shank	27.1	2.50	1.25	10.6	0.94	0.47
Shoulder	26.2	1.03	0.515	10.0	0.78	0.39

DISCUSSION

From the results it was evident that the basement membrane and the epidermis vary in thickness from area to area in the cowhide as shown in Tables I and II. Also, it was observed that as the basement membrane increased in thickness the epidermis increased in thickness, as is shown by the statistical analysis of the thickness measurements given in Table IV. This phenomenon was observed throughout the hide and was not unexpected, for, as mentioned in the introduction, the basement membrane serves as a device for anchoring the epidermis to the dermis, and as the thickness of the epidermis increases, the need for a stronger anchoring structure increases.

The variation in thickness of the epidermis and basement membrane could, and most likely does, have an effect on the unhairing process in leathermaking. The reason for this is quite obvious, for the thickness and the chemical composition of the epidermis and the basement membrane will affect the rate of penetration through these areas by the unhairing agents whether they act

from the grain or flesh sides and will also be factors in determining how strongly the epidermis is anchored to the dermis.

The loss in thickness of the basement membrane and the epidermis in the fixed sections as compared to the unfixed sections can be explained by the shrinking action of the fixatives employed during fixation.

In unfixed sections the thickness of the epidermis varies from 24.92 μ to 32.07 μ, and the thickness of the basement membrane varies from 10.01 μ to 11.71 μ.

In fixed sections the thickness of the epidermis varies from 25.86 μ to 27.37 μ, and the thickness of the basement membrane varies from 10.01 μ to 10.95 μ.

REFERENCES

1. Everett, A., and Cordon, T. *JALCA*, **53**, 548 (1958).
2. Lillie, R. *Histopathological Technique and Practical Histochemistry* (New York: Blakiston Co., 1954), p. 42.
3. Hotchkiss, R. *Archives of Biochem.*, **16**, 131–41 (1948).
4. Pearse, A. *Histochemistry, Theoretical and Applied*, Second Ed. (Boston: Little, Brown and Company, 1960), p. 831.
5. A.S.T.M. *Manual on the Quality Control of Materials* (January, 1951), Table II, p. 63.
6. A.S.T.M. *Standards in Textile Material, Committee D13* (November, 1958), p. 737.